

THIS IS A COPY

2

SECURITY CLASSIFICATION OF THIS PAGE

AD-A228 117

revised
7704-0188

REPORT DOCUMENTATION				1a REPORT SECURITY CLASSIFICATION (U)				1b SECURITY CLASSIFICATION AUTHORITY NA				3 DISTRIBUTION/AVAILABILITY OF REPORT NA			
2a SECURITY CLASSIFICATION AUTHORITY NA				2b DECLASSIFICATION/DOWNGRADING NA				4 PERFORMING ORGANIZATION REPORT NUMBER(S) Rice University				5 MONITORING ORGANIZATION REPORT NUMBER(S) NA			
6a NAME OF PERFORMING ORGANIZATION Rice University				6b OFFICE SYMBOL (If applicable) NA				7a NAME OF MONITORING ORGANIZATION Office of Naval Research				7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000			
6c ADDRESS (City, State, and ZIP Code) Physics Department P.O.Box 1892 Houston, TX 77251				8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research				8b OFFICE SYMBOL (If applicable) ONR				9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0087			
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000				10 SOURCE OF FUNDING NUMBERS		PROGRAM ELEMENT NO 61153N		PROJECT NO RR04108		TASK NO 4414704		WORK UNIT ACCESSION NO			
11 TITLE (Include Security Classification) (U) Studies of Model Ion Channels in Thick Aligned Multilayers of Phospholipids															
12 PERSONAL AUTHOR(S) Huey W. Huang															
13a TYPE OF REPORT Final				13b TIME COVERED FROM 10/1/85 TO 3/31/90				14. DATE OF REPORT (Year, Month, Day) 09/25/1990				15 PAGE COUNT 4			
16 SUPPLEMENTARY NOTATION															
17 COSATI CODES				18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)											
FIELD		GROUP		SUB-GROUP		Uniformly aligned multilayers of membranes; X-ray diffraction, Oriented circular dichroism; Alamethicin; Gramicidin, (JS)									
08															
19 ABSTRACT (Continue on reverse if necessary and identify by block number)															
<p>A uniformly aligned multilayer sample of membranes containing peptides has one-dimensional structural order in which the bilayers are the unit cells and preserves the orientational order of peptides relative to the plane of membrane. The goal of this contract was to develop methods to extract these structural information, and to use such methods to study the structural bases of the voltage-gating mechanisms in model channels. During the contract period, we have 1) developed methods of preparing uniformly aligned multilayer samples of membranes containing peptides and proteins; 2) developed the method of oriented circular dichroism, by which we can indeed extract the orientational information of helical peptides in membrane; 3) found that our multilayer samples produce high resolution diffraction data, from which we can obtain the one-dimensional electron density profiles of peptides in bilayer membranes, in particular the position of heavy atomic ions. We have also successfully use these methods to elucidate the voltage-gating mechanism of the alamethicin channel and determine the location of ion binding sites in the gramicidin channel.</p>															
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS								21. ABSTRACT SECURITY CLASSIFICATION (U)							
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. Igor Vodvanov								22b. TELEPHONE (Include Area Code) 202-696-4056				22c OFFICE SYMBOL ONR			

DD Form 1473, JUN 86

Previous editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

DISTRIBUTION STATEMENT A

(U)

Approved for public release;
Distribution Unlimited

90

10

009

DATE: September 25, 1990

FINAL REPORT ON CONTRACT N00014-86-K-0087
R&T CODE: 4414704

PRINCIPAL INVESTIGATOR: Huey W. Huang
INSTITUTE: Rice University
TITLE: Studies of Model Ion Channels in Thick Aligned Multilayers of Phospholipids
PERIOD COVERED: Oct 1, 1985 to Mar 31, 1990

INTRODUCTION AND OBJECTIVES

Because of the difficulty in making single crystals of membrane ion channels in their native forms (suitable for diffraction studies), there is a lack of structural information for understanding their molecular mechanisms. We believe that, under the circumstances, one-dimensional quasi-crystals of perfect multilayers, in which channels are uniformly oriented within parallel membranes, can provide some of the much needed structural data. Uniformly aligned multilayer membranes have one-dimensional structural order in which the bilayers are the unit cells and contain the orientational order of proteins relative to the plane of membrane. Our objectives are

1. Develop methods of preparing uniformly aligned multilayer samples.
2. Use such samples to produce high resolution x-ray diffraction data.
3. Develop a circular dichroism method to extract the orientational information of peptides or proteins embedded in the multilayers.
4. Use these methods to study model ion channels.

ACCOMPLISHMENTS

1 Multilayer Samples-- We have developed procedures for preparing uniformly aligned multilayer samples of membranes containing peptides or proteins (Huang and Olah, 1987; Olah and Huang, 1988a). For CD and neutron diffraction experiments, the multilayers are aligned homeotropically (lipid bilayers parallel to the substrate surfaces) between two silica plates. The degree of alignment and the phase of lipid can be determined by visual inspection with a polarized microscope. This is possible because the liquid crystalline L_α phase of lipid has unique defect structures, fluidity, and texture different from the gel phase. For x-ray diffraction experiment, the multilayers are aligned between a polished beryllium (Be) plate and a silica plate. In this case the alignment is examined from the silica side by using a reflection polarizing microscope, and x-ray diffraction was measured from the Be side. For electric field experiment, the silica plate is coated with indium tin oxide (ITO) to form a thin transparent electrode on the inside (Olah and Huang, 1988b). Also, for CD experiment, it is important to remove any possible stress in the silica plates. This can be accomplished by temperature annealing at 1150°C for 6 h; the plates are then slowly cooled at a rate of 10°C/h down to 900°C and subsequently at a rate of 100°C/h until room temperature is reached.

The lipids being aligned so far include dilauroyl-, dimyristoyl-, dipalmitoyl-, diphytanoyl-, and dioleoyl- phosphatidylcholine (DLPC, DMPC, DPPC, DPhPC, and DOPC, respectively), dipalmitoylphosphatidylethanolamine (DPPE), L- α -phosphatidylcholine from bovine brain (BBPC) and DMPC-cholesterol mixtures. The peptides and proteins incorporated in multilayer samples include alamethicin, melittin, gramicidin, their synthetic analogues, and cytochrome c (partially aligned).

2. Method of Oriented Circular Dichroism (OCD)--According to the exciton theory of Moffitt (see the review of the theory in Olah and Huang, 1988a), the peptide π - π^* transition in an α -helix is split into perpendicularly and parallel (to the helix axis) polarized components. This important theory is difficult to prove experimentally, because it is difficult to align a sample of α -helices. The use of long polypeptides in an electric field led to conflicting results (see review in Olah and Huang, 1988b), because the bending of long polypeptides was not taken into account. The theory was finally demonstrated experimentally by the use of membrane-spanning α -helices aligned in lipid multilayers; in particular, it was shown that the CD band of helices at 205 nm is polarized along the axis (Olah and Huang, 1988a; 1988b).

This theory is clearly usable to determine the orientation of α -helices in a membrane. Thus we developed a method of oriented circular dichroism (OCD), in which the CD spectra of a multilayer sample were measured with light incident at various angles (called α) with respect to the normal of the membrane planes (Wu et al., 1990). For example, we have found two different sets of OCD for a multilayer sample of alamethicin in DPhPC in two different hydration conditions. (The CD of alamethicin, when it is associated with membrane, is closely that of a typical α -helix; however, its amplitude indicates that only 60-70% of the residues are helical.) We have shown (Wu et al., 1990) from the angular (α) dependence that one set of OCD (spectra I) represents α -helices oriented perpendicularly to the plane of membrane, whereas the other set (spectra S) represents α -helices oriented parallel to the plane of membrane. Furthermore, the analysis showed that spectra I and S are related by a simple rotation, indicating that there is no change of the secondary structure between two orientations.

It is well known that measurement of CD can be distorted by the effects of linear dichroism and birefringence; and these effects can be serious when a multilayer sample is measured at an oblique angle. Therefore, we first analyzed the CD artifacts theoretically and demonstrated the effects step by step with a tilted sample. We then designed a special sample chamber and worked out a measurement procedure to completely remove the spurious effects from the OCD spectrum (Wu et al., 1990).

3. X-ray Diffraction--To demonstrate the feasibility of using x-ray diffraction to study membrane peptides, we measured the diffraction patterns of gramicidin-DLPC multilayers with and without ions (Ti^+ , K^+ , Ba^{++} , Mg^{++}). The idea is to use the difference electron density profiles to reveal the ion binding sites or the ion distributions in the gramicidin channel.

Although membrane diffractions have been studied since the late 60's, our method of using single-domain multilayers and the θ - 2θ scan geometry is new. (Neutron diffractions usually employ the θ - 2θ scan geometry, but their resolutions are low, due to low fluxes and the incoherent scattering of hydrogen.) That means, first of all, we have to show that the new method can faithfully reproduce a known result. This was tested on the DMPC-cholesterol system, for which Frank and Lieb (1979) had obtained a high-resolution electron density profile. We used the H_2O swelling method to determine the phases like all previous methods. We found that because we have a well-defined sample geometry, the data reduction is straightforward and rigorous, including (1) background subtraction, (2) corrections for polarization, the Lorentz factor, scattering volume, Be and specimen absorption, the second harmonic (which becomes significant due to the absorption by the Be plate) and the atomic scattering factors, and (3) the detector vertical slit correction for beam divergence and sample mosaic (0.3° - 0.5°). Our results, including 13 Bragg orders, are in complete agreement with a previous result (Olah, Ph.D. thesis 1990).

<input checked="checked" type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

Codes

Dist and/or Special

A-1

From the difference electron density profiles of gramicidin-DLPC bilayers with Tl^+ , with K^+ , and without ions, two symmetrical binding sites of thallium ion are found at 9.6 Å from the center of the channel. Similarly Ba^+ binding sites are located at the mouth of the channel, 10.3 Å from the center. Also, from the diffraction patterns of DLPC multilayers with and without gramicidin, we have obtained the electron density profile of the gramicidin channel. study alamethicin and gramicidin.

4. Voltage Gating Mechanism of Alamethicin Channel--Although the voltage-dependent alamethicin channel is one of the best characterized ion channels, so far no agreement has been reached about which model best describes all the experimental data. While the barrel-stave configuration is accepted by most investigators as a good description of the conducting state of alamethicin, there are conflicting reports on its nonconducting state--in the absence of an applied field, some found alamethicin molecules on the membrane surface, but others found them incorporated in the hydrophobic core of the membrane. This problem is now resolved by the discovery of a phase transition of alamethicin in membrane. We have discovered that, as a function of lipid/peptide ratio L/P and the chemical potential of water μ , alamethicin molecules are either all bind parallel to the membrane surface or all insert perpendicularly into the membrane. The state of alamethicin was monitored by the method of oriented circular dichroism, using aligned multilayer samples in the liquid crystalline L_α phase. If L/P exceeds a critical value, all peptide molecules are on the membrane surface. If L/P is below the critical value, all peptide molecules are incorporated in the membrane when μ is high; when μ is low, alamethicin is again on the membrane surface. In a typical conduction experiment, alamethicin molecules are partitioned between the aqueous phase and the lipid phase; in the lipid phase, the lipid/peptide ratio is such that all alamethicin molecules are on the membrane surface in the absence of a field. When an electric field is applied, it is those surface peptide molecules (rather than those in the aqueous phase) which will probabilistically turn into the membrane to form channels. The phase transition is a manifestation of membrane-mediated intermolecular interactions between peptide molecules. It can be qualitatively explained in terms of a model (Huang and Wu, 1990).

5. Location of Ion Binding Sites in the Gramicidin Channel--This is the first x-ray diffraction on gramicidin in its membrane-active form. High-resolution Bragg reflections of uniformly aligned multilayer samples of membranes containing gramicidin and ions (Tl^+ , K^+ , Ba^{++} , Mg^{++} or without ions) are obtained. From the difference electron density profiles, we found a pair of symmetrically located ion binding sites for Tl^+ at $9.6 \pm 0.3 \text{ Å}$ and for Ba^{++} at $13.0 \pm 0.2 \text{ Å}$ from the midpoint of the gramicidin channel. The location of Ba^{++} binding sites is near the ends of the channel, consistent with the experimental observation that divalent cations do not permeate but block the channel. The location of Tl^+ binding sites is somewhat a surprise. It was generally thought that monovalent cations bind to the first turn of the helix from the mouth of the channel. (It is now generally accepted that the gramicidin channel is a cylindrical pore formed by two monomers, each a single-stranded $\beta^{6.3}$ helix and hydrogen-bonded head-to-head at their N-termini.) But our experiment shows that the Tl^+ binding site is either near the bottom of or below the first turn of the helix. (Olah, Huang, Liu, and Wu, 1990)

PUBLICATIONS

H. W. Huang, "Deformation Free Energy of Bilayer Membrane and Its Effect on Gramicidin Channel Lifetime," *Biophys. J.* 50,1061-1071 (1986).

- T. Y. Teng and H. W. Huang, "Hemoglobin and Myoglobin Embedded in Dry Polyvinyl Alcohol Film for X-ray Absorption Studies." *Biochim. Biophys. Acta* 874,13-18 (1986).
- H. W. Huang and G. A. Olah, "Uniformly Oriented Gramicidin Channels Embedded in Thick Monodomain Lecithin Multilayers " *Biophys. J.* 51, 989-992 (1987).
- H. W. Huang, T. Y. Teng and G. A. Olah, "5K Extended X-ray Absorption Fine Structure and 40K 10-second Resolved Extended X-ray Absorption Fine Structure Studies of Photolyzed Carboxymyoglobin," *Biochemistry* 26, 8066-8072 (1987).
- H. W. Huang with G. A. Olah, "Circular Dichroism of Oriented α -helices. I. Proof of the Exciton Theory," *J. Chem. Phys. Phys.* 89, 2531-2538 (1988).
- H. W. Huang with G. A. Olah, "Circular Dichroism of Oriented α -Helices. II. Electric Field Oriented Polypeptides, *J. Chem. Phys.* 89, 6956-6962 (1988).
- Y. Wu, H. W. Huang, and G. A. Olah, "Method of Oriented Circular Dichroism." *Biophys. J.* 57, 797-806 (1990)
- G. A. Olah, H. W. Huang, W. Liu and Y. Wu, "Location of Ion Binding Sites in the Gramicidin Channel by X-ray Diffraction" *J. Mol. Biol.* submitted.
- H. W. Huang and Y. Wu, "Alamethicin-Membrane Interactions and Voltage-gating Mechanism of Alamethicin Channel." *Biophys. J.* submitted.

Annual Final and Technical Reports

ADMINISTRATORS

Dr. Igor Vodyanoy, Code 1141SB (2 copies)
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies) (Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Administrative Contracting Officer
ONR Resident Representative
(address varies - obtain from contract or
your business office)

Dr. Robert J. Nowak, Code 1113ES
Scientific Officer, Electrochemical
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager
Biological/Human Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager Defense Technical
Support Technology Directorate
Office of Naval Technology, Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Annual and Final Reports Only (one copy each)

DoD ACTIVITIES

Commander
Chemical and Biological Sciences Division
Research Army Research Office, P. O. Box 1221
Research Triangle Park, NC 27709

Directorate of Life Sciences
Air Force Office of Scientific
Bolling Air Force Base Research
Washington, DC 20332

Head
Biomolecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375

Final and Technical Reports Only

Director, Naval Research Laboratory (6 copies)
Attn: Technical Information Division, Code 2627
Washington, DC 20375